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CASE REPORT

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Corneal cross-linking as a treatment for corneal dystrophy with secondary bacterial infection in a Friesian horse

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Abstract

Corneal cross-linking should be considered as treatment option in Friesian horses with infectious keratitis and corneal dystrophy. Optical coherence tomography, giving information of corneal structure, can help for diagnosis and monitoring.

KEYWORDS

corneal ectasia, corneal ulcer, OCT, PMD, progressive corneal disorder, stromal loss

1 | INTRODUCTION

Corneal dystrophy in Friesian horses was described as a variant of pellucid marginal degeneration (PMD) in humans. Since corneal cross-linking (CXL) has been beneficial in the stabilization of PMD, a typical ulcerative corneal lesion with secondary bacterial infection in a Friesian horse was successfully CXL treated.

Corneal dystrophies are defined as a group of bilateral, genetically determined, noninflammatory corneal diseases. They can be divided in three groups according to anatomic location: epithelial, stromal or endothelial.[1,2] In humans, pellucid marginal degeneration (PMD) is a bilateral progressive corneal disorder characterized by a noninflammatory ventral band of corneal thinning and ectasia.[3] It is considered a variant of keratoconus.[4] Corneal ectasia results in astigmatism, which leads to impaired vision. Multiple treatment modalities are currently used, including intracorneal rings, contact lenses or keratoplasty.[5] Corneal thinning can lead to corneal perforation which must be treated surgically.[6]

Corneal dystrophy in Friesian horses is characterized as a bilateral symmetric corneal stromal loss without signs of inflammation.[7] Middle aged and male horses are overrepresented.[8] No signs of uveitis are typically present unless corneal perforation occurs. The disease was suggested to represent a variant of PMD in a recent publication.[7]

Optical coherence tomography (OCT) is an important noninvasive imaging tool for corneal diseases in human ophthalmology and widely used in animal research.[9-14] It generates cross-sectional images providing information with resolutions approaching that of histopathology.[15] Progression of both the initial corneal disease and the healing process following treatment could be visualized very well in our patient with this technique.

Corneal cross-linking (CXL) is performed as a minimally invasive procedure for stabilization of corneal ectatic disorders and to improve functional visual acuity in humans.[16-18] In the normal cornea, there are covalent bonds between collagen molecules. The process of corneal cross-linking increases the number of these bonds by photopolymerization. Riboflavin is applied to the cornea and activated by UV-A light (370 nm), which creates covalent bonds between protein residues and/or other molecules.[19] As a result, CXL improves corneal stromal resistance to enzymatic digestion[20-24] and damages multiple targets within microorganisms, thus eliminating them.[25-28] Corneal cross-linking was proven to be effective against bacteria in vitro and in vivo and is widely used in infectious keratitis.[29-34] If used to treat infectious keratitis and associated corneal stromal degradation, CXL is referred to as PACK-CXL, meaning *Photo Activated Chromophore for Keratitis—Corneal Cross-linking*.[31,35,36] Corneal

cross-linking was successfully used to stabilize an ulcerative corneal lesion with stromal loss in a Friesian horse in this case report.

2 | MATERIALS AND METHODS

2.1 | Ophthalmic examination

A complete ophthalmic examination including slit lamp biomicroscopy (SL-17; Kowa), fluorescein testing (Fluostrip; Contacare Ophthalmics & Diagnostics), indirect ophthalmoscopy (HEINE Omega 500, Heine Optotechnik GmbH & Co. KG); and tonometry (Tonovet®; Icare Finland Oy) was performed during the initial and each recheck examination. A detailed examination of the corneal lesions was performed with OCT (Envisu R2210 with 20 mm telecentric anterior segment lens; Bioptigen, Leica microsystems).

2.2 | Corneal cross-linking

A 0.1% riboflavin/ 1% hydroxypropyl methylcellulose solution without dextran (Peschke M®; System Vision SA) was applied to the cornea every 1-2 minutes for 20 minutes followed by an accelerated CXL treatment (CCL Vet® corneal cross-linking system, Peschke Trade GmbH). A total dose of irradiation energy (fluence) of 10.8 J/cm^2 was delivered to the treatment area via two cycles of 45 mW/cm^2 UV-A irradiation for 2 minutes. Two drops of riboflavin were applied between the cycles. Saturation of the cornea with riboflavin and the CXL treatment were both performed with the horse standing and sedated.

3 | CASE DESCRIPTION

A 16-year-old, male castrated, Friesian horse was presented to the Ophthalmology section at the Vetsuisse Faculty of the University of Zurich because of reported chronic conjunctivitis on the left side and acute bilateral fluorescein-negative changes in the cornea with blepharospasm on the left eye. The horse had been treated with diclofenac eye drops on both eyes once daily for two days.

4 | CLINICAL FINDINGS AND TREATMENT

Both eyes were open with mild mucous discharge. Intraocular pressure was 16 mm Hg in the right eye and 12 mm Hg in the left eye. A ventro-temporally located, focal, oval gray to white corneal opacification was identified in both eyes. A fluorescein-negative corneal facet of about 7 mm diameter with ±20% stromal loss in the center of the facet was detected on the right eye. Optical coherence tomography examination revealed a corneal defect with 15% stromal loss and hyperreflective subtending stroma, covered by an irregular and centrally thinned but intact corneal epithelium (mean stromal thickness of surrounding unaffected area 567 ± 42 µm) (Figure 1A,B). The corneal opacity on the left eye was 1 cm in diameter with a somewhat irregular fluorescein-negative epithelial surface in the center of the lesion. Optical coherence tomography examination revealed an intact but hyperreflective epithelium with irregular surface and mildly hyperreflective subtending stroma (Figure 1C,D). Findings were characteristic of Friesian corneal dystrophy.[7] Due to an improvement in comfort observed during the two previous days, the topical diclofenac treatment initiated by the referring practitioner was continued twice daily on both eyes.

Five days later, the horse returned as an emergency case because of moderate blepharospasm and purulent ocular discharge on the right eye. The corneal lesion was now fluorescein positive with ±20% stromal loss, stromal infiltrates were present throughout the ulcerated area and cytology revealed epithelial cells, neutrophils and rod-shaped bacteria. Optical coherence tomography demonstrated epithelial loss, stromal hyperreflectivity and changes in stromal organization. Bacterial culture results were negative. The pupil was miotic (Figure 2). Medical treatment was changed to topical ofloxacin (Floxal® eye drops, Bausch & Lomb Swiss AG), heterologous serum and EDTA 0.33% eye drops (compounded, Kantonsapotheke Zurich) every three hours, atropine 1% eye drops (Minims® atropine sulfate 1.0% w/v eye drops, Bausch & Lomb) twice daily and systemic flunixin (Fluniximin® ad us. vet., Dr E. Graeub AG) 1 mg/kg intravenously twice daily.

No improvement in clinical signs was observed the following day. Since corneal dystrophy, with typical findings before ulceration, was thought to be the underlying cause for the corneal infection, corneal cross-linking was performed as described above with the horse in standing sedation with detomidine (Medesedan®, Virbac Switzerland AG) 10 mcg/kg intravenously. The ulcer stabilized during the following two days, presenting without additional stromal loss, a more stable-looking stroma, decreased density of infiltrates, progressive epithelization and decrease in blepharospasm. Treatment was changed to topical ofloxacin, serum and EDTA 0.33% eye drops every 6 hours, atropine 1% eye drops every other day and oral flunixin (Equinixin®, Norbrook Laboratories) 1.1 mg/kg once daily. The horse was discharged after 4 days with the topical therapy described above and oral meloxicam once daily (Inflacam®, Virbac Switzerland AG; 0.6 mg/kg).

The ulcer epithelialized within the three weeks following CXL treatment without additional loss of corneal stroma and the frequency of topical serum and EDTA therapy was gradually reduced during this period of time. Oral meloxicam was discontinued two weeks after CXL. A stable epithelialized corneal scar with 20% stromal loss had developed on the right eye one month after CXL therapy. Optical coherence tomography demonstrated a smooth, intact but hyperreflective epithelium with irregular surface and a highly disorganized and hyperreflective subtending corneal stroma (Figure 3C,D). Three months after CXL therapy a slightly elevated vascularized pannus had filled the epithelialized corneal scar site. Optical coherence tomography demonstrated a smooth, intact, regular epithelium of normal reflectivity and an organized, well demarcated, highly reflective subtending stroma (Figure 3E,F). The left eye was stable during follow-up.

5 | DISCUSSION

Treatment with corneal cross-linking in combination with medical therapy was associated with prevention of progressive corneal stromal loss in a Friesian horse with infectious keratitis and underlying corneal dystrophy as presented in this case report.

Corneal ectatic disorders in humans are characterized as a stromal instability originating from collagen abnormalities.[19] The etiology of keratoconus is not fully understood, but it can occur as a result of genetic predisposition triggered by environmental factors.[37] Between 8% and 10% of patients have a hereditary component and family history.[37] The pathogenesis may be associated with contact lens wear, eye rubbing, Down's syndrome, atopic disease or connective tissue disorders like Ehlers-Danlos syndrome, Marfan syndrome or Osteogenesis Imperfecta.[37,38] The

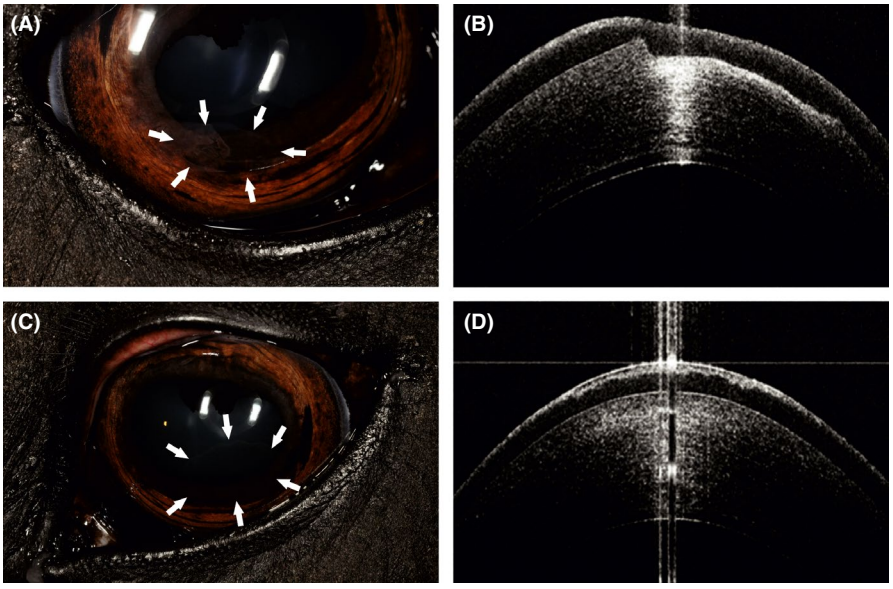

FIGURE 1 A, Picture of the cornea of the right eye (OD) with inconspicuous ventro-temporally located oval corneal opacification (arrows). About 20% stromal loss was identified with the slit lamp. B, Correlating OCT image demonstrating an intact epithelium with loss and hyperreflectivity of the subtending stroma. C, Picture of the left eye (OS) with a very mild ring-shaped corneal opacification located in the ventro-temporal region (arrows). D, Superficial hyperreflectivity in an intact epithelium with near normal to mildly hyperreflective subtending stroma visible on the correlating OCT image

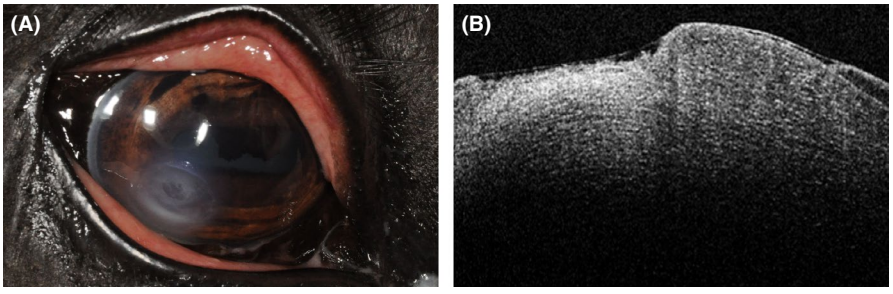

FIGURE 2 A, Clinical picture of OD at presentation five days later with an epithelial defect in the previously identified area of 20% stromal loss and stromal infiltrates throughout the ulcerated area. The pupil was miotic at presentation. B, Correlating OCT image revealing loss of epithelium, stromal hyperreflectivity and changes in stromal organization

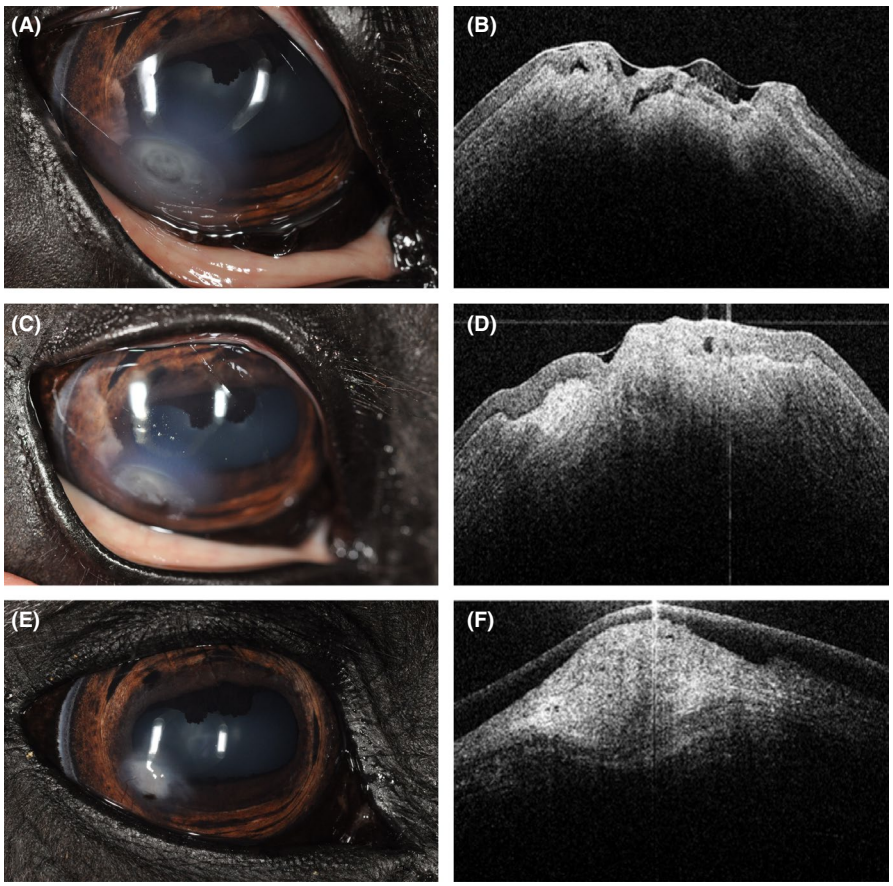

FIGURE 3 A, Picture of OD one week after CXL. B, The correlating OCT image shows a newly formed thin and irregular epithelium. C, Picture of OD one month after CXL. D, OCT demonstrated a smooth, intact but hyperreflective epithelium with irregular surface and a highly disorganized and hyperreflective subtending corneal stroma. E, Picture of OD three months after CXL displaying the healing process. A vascularized stromal scar with intact epithelium is now visible. F, Correlating OCT image demonstrating an intact and smooth epithelium of normal reflectivity and an organized, well demarcated, highly reflective subtending stroma. A parallel pattern is again visible in the deeper sections of the corneal stroma

biosynthesis of extracellular matrix components like proteoglycans is altered in the disease, leading to a decrease in keratan sulfate chains and an increase in dermatan sulfate chains. This change in glycosaminoglycan (GAG) ratio weakens the cohesive forces of collagen sheets.[39] Changes in proteoglycan-GAG composition in the stroma of scarred keratoconus corneas were also reported.[40] Corneal samples of PMD patients contained degenerated collagen fibrils and very large proteoglycans.[41]

A multisystem manifestation of improper collagen formation has been hypothesized as an underlying cause for bilateral corneal stromal loss in Friesian horses with corneal dystrophy as well. As mostly male individuals are affected, a simple X-chromosomal inheritance was suggested but could not be proven.[8] Collagen abnormalities have been described in other organs in Friesian horses. For example, increased matrix metalloproteinase activity, lysine hydroxylation and elastin cross-linking were found at the site of aortic rupture in affected horses.[42] Another study described disorganized elastin in the aorta of affected animals.[43] More abundant and abnormal collagen between muscular layers was described in Friesian horses with esophageal dysfunction.[44] Results of a recent study indicate a higher rate of collagen degradation in the Friesian horse, which could explain the general predisposition to connective tissue disorders.[45] Bacterial infection developed secondary to corneal stromal degeneration and disruption of the epithelium in the Friesian horse presented in our case report. Previous topical diclofenac treatment could have been a contributing factor as it is reported to be associated with corneal ulceration.[46] Infected corneal ulcers are a common, painful and potentially blinding disease in all species[47] usually caused by secondary bacterial or fungal infections in horses.[47-49] The inflammatory response to infection activates proteolytic collagen-dissolving enzymes in the corneal stroma resulting in "corneal melting" which can lead to corneal ulcer deepening, corneal perforation and loss of vision despite aggressive medical therapy.[47,50]

Corneal perforation appears frequently in Friesian horses with corneal dystrophy, even without secondary infections, and surgical repair is often needed to save the eye.[7] Surgical intervention usually necessitates general anesthesia with a ~1% risk of anesthesia-related death in horses, which is much higher than in dogs and cats.[51] Additionally, the frequency of dehiscence of conjunctival grafts was high in Friesian horses with corneal dystrophy (5/9 eyes) with ongoing degenerative processes as presumed cause and a second anesthesia necessary in three out of five eyes.[7] Alternatives to surgical interventions under general anesthesia would, therefore, be welcome, especially since these patients can represent an increased anesthetic risk as a result of associated cardiovascular disease.[42,43]

Corneal cross-linking has been described as treatment alternative for pellucid marginal degeneration in human patients[52-54] and is a cost-effective treatment modality that can be performed on a sedated standing horse,[55] provided that the disease is detected early when corneal stromal loss is still limited.[56] A minimum corneal thickness of 400 μm to allow sufficient absorption of the UV-A radiation in the riboflavin saturated stroma is recommended to safely perform CXL and avoid endothelial, lens and retinal damage.[19] Historically, single fluence protocols delivering 5.4 J/cm^2 of energy to the corneal stroma have been used for the treatment of keratoconus and bacterial keratitis.[29,32,57] A high energy double fluence CXL protocol (10.8 J/cm^2) was used in this case since single fluence protocols have demonstrated somewhat variable effectivity in horses with infectious keratitis.[55] Also, increasing fluence increases treatment effect.[58-60] Moderate fluence (7.2 J/cm^2) significantly increased corneal stromal resistance to enzymatic digestion ex vivo compared to corneas cross-linked with routine fluences (5.4 J/cm^2).[59] In addition to that, large CXL fluence increases from 5.4 to 27 J/cm^2 caused large increases in antibacterial efficacy. Bacterial killing rates increased from 50% to >90% with double fluences of 10.8 J/cm^2 and to 100% with triple fluences of 16.2 J/cm^2 and above.[58,60] Increasing corneal stromal resistance to enzymatic digestion and killing of bacteria were both desirable treatment effects because of the presumed enzymatic corneal stromal degeneration caused by the primary corneal dystrophy and secondary infectious keratitis in our patient.[47,50]

Significant CXL side effects are unlikely to occur with a double fluence protocol since topography-guided CXL protocols with regional fluences ranging from 7.2 to 15 J/cm^2 have been used for human keratoconus patients without detrimental effects.[61] Also, no negative effects on the corneal endothelium or retina were reported following the use of 15-20 J/cm^2 fluences in preclinical studies on nonhuman primates and 10-15 J/cm^2 fluences in a clinical safety study on blind human eyes.[62]

Optical coherence tomography is a valuable diagnostic tool in PMD patients and was helpful to determine epithelial integrity and the exact remaining corneal thickness as stromal loss was slightly overestimated via slit lamp examination.[63,64]

The medical ulcer treatment likely contributed to the positive treatment outcome in the case described here. However, ulcer healing was reported to last from six to eight weeks in Friesian horses treated for corneal dystrophy,[7] which is twice the time that the Friesian horse in our report needed for complete reepithelization. However, this might not be a fair comparison since only one eye was treated and the disease was detected and treated early in our case.

In conclusion, corneal cross-linking seemingly stabilized the cornea in the case presented here and should, therefore, be considered as treatment option for Friesian horses with infectious keratitis and underlying corneal dystrophy.

The efficacy of CXL for stabilization of the corneal stroma and prevention of corneal perforation in Friesian horses with corneal dystrophy will need to be evaluated in a multicenter proof of concept study.

CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTION

CC: Acquisition and interpretation of data, drafting the manuscript. SAP: Acquisition and interpretation of data, revising the manuscript. AL: Acquisition of data. KV: Substantial contributions to conceptions and design, acquisition and interpretation of data, revising the manuscript.

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